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## HYDROGEN-ION STUDIES

### VI. HYDROGEN-ION CHANGES ON PRECIPITATION OF HUMAN SERUM BY IMMUNE SERUM \*

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Proteins are regarded, generally, as amphoteric electrolytes, that is, substances which are able to unite with acid as well as alkali.<sup>1</sup> The hydrogen-ion concentration of the surrounding solution determines whether they unite with the one or the other. When the hydrogen-ion concentration of the solution exceeds a critical point which is known as the iso-electric point of the protein, the protein combines with acid to form a salt which, dissociating, gives rise to a protein cation carrying a positive electrical charge and an acid anion with a negative electrical charge. When the hydrogen-ion concentration of the solution is on the alkaline side of the iso-electric point of the protein, the protein combines with metals to form a salt such as sodium proteinate which dissociates into negatively charged protein ions, and positively charged ions of the metal.

That precipitation of a colloid bearing an electrical charge of one sign is accomplished by the addition of a colloid bearing an opposite charge, and that both colloids are carried down in the precipitate, is generally recognized. The precipitin reaction on mixing an antigen in solution with its homologous immune serum probably belongs to the same group of reactions. Biltz<sup>2</sup> suggests the following rules regarding the precipitation of one colloid by another when mixed together quickly and uniformly:

If to a given colloidal solution, one of the opposite sign is added in small proportion, there is no precipitation. As the quantity of the second increases the coagulative action follows parallel until a proportion is reached which causes immediate coagulation. As the amount is still further increased, coagulation ceases; that is, there is an optimum precipitation for certain proportions, and when these favorable proportions are exceeded on either side, no precipitation occurs.

Changes in the hydrogen-ion concentration of a medium frequently occur with the chemical interaction of the substances contained, and

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<sup>1</sup> Loeb, Jacques: Jour. Gen. Physiol., 1918-19, 1, pp. 39, 237, 363, 483, 559.

<sup>2</sup> Berichte d. Deutsch. Chem. Gesellschaft, 1904, 37, p. 1095.

these changes in reaction are important in understanding the nature of the chemical change. Previous studies<sup>3</sup> of the hydrogen-ion changes on agglutination of bacteria by homologous immune serum, and of the changes in reaction on the precipitation of a colloidal gold solution by spinal fluid (Lange test) demonstrated that these chemical reactions are accompanied by an increase of the hydroxyl-ion (alkalinity) content of the medium. Since both the bacteria in a suspension in salt solution and the colloidal gold particles of the gold solution carry negative electrical charges, it is likely that their agglutination or precipitation are similar chemical reactions, that the changes are governed by well-known laws obtaining in the precipitation of a colloid bearing an electrical charge of one sign by the addition of a colloid with an opposite electrical charge, and that the precipitate contains both the precipitating and the precipitated colloids. The precipitin reaction is analogous, at least to a certain extent, with these reactions, and therefore experiments were made to determine whether similar changes occur in the hydrogen-ion concentration of the medium.

Human serum and homologous immune rabbit serum were used in these experiments, the amounts in c c being as follows:

				Control
Immune rabbit serum.....	0.1	0.1	0.1	0.1
Human serum.....	0.1	0.05	0.025 etc.	0
NaCl, 0.9%.....	1.8	1.85	1.875	1.9
Normal rabbit serum.....	0.1	0.1	0.1	0.1
Human serum.....	0.1	0.05	0.025 etc.	0
NaCl, 0.9%.....	1.8	1.85	1.875	1.9

The dilutions were made in clean, sterile, glass test tubes with precautions against bacterial contamination, and then kept at icebox temperature over night, the reactions of the tubes being determined after about 18 hours, according to the gas-chain method, at a constant temperature (25 C.).

Table 1 gives the results of one experiment which are like those obtained in others. The experiments demonstrate that there is an increase of the hydroxyl-ion content of the medium with the precipitation of human serum by homologous immune rabbit serum. The changes in reaction are similar to those on agglutination of bacteria by homologous immune serum, and on precipitation of colloidal gold solution by spinal fluid (Lange test). When plotted they form curves like those in the other studies.<sup>3</sup> The reactions of the control series fall in a straight line like a graph of hydrogen-ion concentrations obtained by similar dilutions of two chemically inert solutions with different reactions. The inhibition of precipitation in the first antigen-

<sup>3</sup> Jour. Infect. Dis., 1922, 30, pp. 259, 263.

antibody dilution corresponds with observations already well known and probably results from an excess of antigen in the mixture.

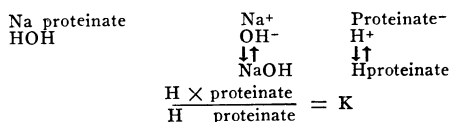
TABLE 1  
RESULTS OF EXPERIMENT

Dilution of Human Serum	Immune Rabbit Serum		Normal Rabbit Serum	
	P <sub>H</sub>	Precipitation	P <sub>H</sub>	Precipitation
1:20.....	8.08	Turbid only	7.90	0
1:40.....	7.98	+++	7.84	0
1:80.....	7.93	+++	7.74	0
1:160.....	7.85	++	7.74	0
1:320.....	7.82	++	—	—
1:640.....	7.80	++	—	—
1:1280.....	7.76	+	—	—
Control.....	7.74	0	7.74	0
Salt solution				

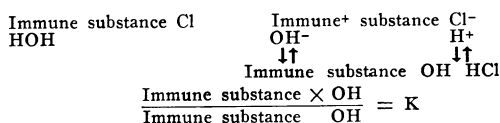
Each tube contains 1/20th volume rabbit serum.

#### COMMENT

Since the results obtained with the precipitation test are like those obtained with the agglutination of bacteria, it is fair to regard the two phenomena as chemically similar. The presence of an inorganic salt such as sodium chloride is as essential for precipitation in the precipitin test as it is for the agglutination of bacteria by homologous immune serum. Bacteria in sodium chloride solution carry negative electrical charges; that is, they are ionized as the acid anion of a salt. When they are added to a sodium chloride solution, the reaction of the medium becomes more alkaline, a change similar to that observed when a salt of a strong base and a weak acid is added to water. This alkalinity results because of dissociation changes of the salt and water in establishing their equilibrium and in satisfying their dissociation constants. The protein substance or substances in human serum concerned with the precipitin reaction may be represented as dissociating in salt solution according to the formula:



and the immune substance in the homologous serum according to the formula:



The mutual precipitation of colloids bearing opposite electrical charges is generally known, and the laws governing this chemical action probably apply equally well in the precipitin reaction.

The increase of hydroxyl ions on the precipitation of human serum by homologous immune serum probably results from a liberation of the base (Na) whose dissociation constant is greater than that of the immune substance.

#### SUMMARY

The precipitation of human serum by homologous immune serum is accompanied by an increase in the alkalinity of the medium similar to that observed on the agglutination of bacteria by immune serum and on the precipitation of colloidal gold by spinal fluid (Lange test).